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Abstract

Melanoma has always been described as an immunogenic tumor. Until 2011 the standard of care in metastatic melanoma was chemotherapy, with response rates between 15-20% and without any benefit on survival. Melanoma was the first cancer model to introduce the immune-checkpoint inhibitors in clinical practice. In this review the preclinical bases and the main clinical studies that led to the approval of the immunotherapy agents will be described with insights on combination of immunotherapies of combination and on predictive biomarkers of benefit from immunotherapy.

Keywords

Melanoma, Immunotherapy, Immune-checkpoint inhibitors, CTLA-4, PD-1, Biomarker.

Introduction

Melanoma has always been described as an immunogenic tumor. Despite that, until 2011 the standard of care in metastatic melanoma was chemotherapy (ChT), with response rates ranging between 15-20% and no benefit on overall survival (OS). The only available immunological therapy was high-dose interleukin-2 (IL-2), which could induce long-lasting responses in a small subset of patients; however, high-dose IL-2 was associated with a high rate of severe toxicities[1].

Immune checkpoint inhibitors (ICI) were the first class of therapy shown to improve the overall survival for patients with advanced melanoma, and the anti-CTLA-4 antibody ipilimumab and the anti-PD-1 antibodies nivolumab and pembrolizumab are now the standard of care in everyday clinical practice[2]. In this review, the preclinical bases and the main clinical studies that led to the approval of such immunotherapy agents currently used in clinical practice will be described, with insights on combination immunotherapy and important future challenges: the treatment of brain metastases and the pursuit of predictive biomarkers.

Anti-CTLA-4 antibodies

To be activated, T lymphocytes must recognize an antigen exposed on the surface of the antigen presenting cells (APCs) through the major histocompatibility complex (MHC). The interaction between the T cell receptor (TCR) and the MHC represents the signal 1 of the activation process[3] (Figure 1).

However, T lymphocytes require a second signal (signal 2) to complete their activation. This signal derives from the interaction between the CD28 co-stimulatory receptor and the CD80 and CD86 molecules (also known as B7.1 and B7.2), expressed on the surface of APCs cells. The peculiarity of signal 2 is that the interaction of CD28 can be displaced by another receptor expressed on the surface of the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). This is an inhibitory receptor and, besides, it has greater affinity for the B7 molecules than CD28[3].

From a physiological point of view, CTLA-4 is essentially involved in the reduction of the T helper cells (Th) activity and in the strengthening of regulatory T cells (T_{Regs}), a sub-category of T lymphocytes which is strictly involved in the maintaining self-tolerance and where CTLA-4 is constitutively expressed[3].

CTLA-4 is a target gene of the Forkhead box P3 transcription factor (FOXP3), a crucial factor in the genesis of T_{Regs} cell lineage[4]. The role of CTLA-4 and the function of T_{Regs} is closely related. In fact, persons bearing the homozygous mutation in *FOXP3* present the clinical signs of a particular autoimmune hereditary syndrome, known as IPEX, an X-linked inheritance disease, which includes: immune dysregulation, polyendocrinopathy and enteropathy[5]. These clinical manifestations are very close to the main side effects of the anti-CTLA-4 antibodies observed in clinical studies and in clinical practice.

The inhibition of CTLA-4 is a therapeutic strategy which is therefore based on both the enhancement of the T CD4 effector lymphocytes and the inhibition of T_{Regs} lymphocytes.

Allison and colleagues showed that despite this immune-checkpoint was not tumor-specific there could still be a rationale for a clinical study with an anti-CTLA-4 agent in some immunogenic tumors.

Tremelimumab and ipilimumab were the first fully humanized anti-CTLA-4 antibodies that were clinically tested in 2000.

In the case of tremelimumab, a phase 3 randomized clinical trial showed no benefit in terms of survival compared to dacarbazine, with a high rate of immune-related adverse events (irAEs)[6].

On the other hand, for ipilimumab, less toxic doses were studied and the algorithms for the management of immunological toxicities have been improved, resulting in an overall reduction of mortality and morbidity, and in an advantage in terms of survival in melanoma. In particular, two phase 3 clinical studies (CA 184-002 and CA 184-024) led to the approval of ipilimumab by U.S. Food and Drug Administration (FDA).

In CA 184-002, a total of 676 previously treated patients were randomized (in a 3:1:1 ratio) to receive ipilimumab 3 mg/kg + gp100 (Melanoma Peptide Vaccine) *vs.* ipilimumab 3 mg/kg + placebo *vs.* gp100 alone. Ipilimumab showed an advantage in terms of median overall survival (OS) of 3.6 months: the median OS was 10.1 months among patients in the ipilimumab alone arm (HR for death in comparison with gp100 alone 0.66; p=0.003), with no difference in OS between the ipilimumab arms (HR 1.04; p=0.76); median OS was 6.4 months in the gp100 vaccine alone group[7,8].

The CA184-024 study compared ipilimumab 10 mg/kg + dacarbazine (DTIC) *vs.* DTIC alone in naïve patients. Ipilimumab showed a significant improvement in terms of survival rates at 3 years (20.8% *vs.* 12.2% in the ipilimumab + DTIC and DTIC alone arms, respectively). HR for death in the ipilimumab + DTIC treatment arm was 0.72; p=0.001[9].

Ipilimumab led to a significant advantage in term of clinical response and OS in patients with metastatic melanoma, but irAEs associated with such treatment are a challenging limit in clinical practice. CTLA-4 is an important immune check-point implicated in the maintenance of immunologic homeostasis, and when blocked, it can induce immune-toxicity. The most frequently observed ipilimumab-induced irAEs are: rashes, colitis, hepatitis and endocrinopathies. In particular, a difference has been observed between the 3 mg/kg dose group and the 10 mg/kg dose. Treatment-related serious AEs were 37% *vs.* 18% (in the 10 mg/kg and in the 3 mg/kg dose, respectively), with four (1%) *vs.* two (<1%) treatment-related deaths. The 3 mg/kg dose showed to be more favorable in terms of safety, with no loss in terms of efficacy[10]. However, even if the FDA approved dose is now 3 mg/kg, a lower dose should still be experimented in order to create a more suitable balance between efficacy and safety, in particular when used in combination with other agents.

Anti-PD-1 antibodies

PD-1 receptor has a consistent action on the immuno-escaping mechanisms within the tumor microenviroment[3]. PD-1 is an immunological checkpoint inhibiting the lymphocyte activity in the peripheral tissue when the immunological specific response has already been established, unlike CTLA-4 which is involved during the T lymphocyte activation phase[3]. The PD-1 ligands are PD-1 ligand 1 (PD-L1, also known as B7-H1) and PD-1 ligand 2 (PD-L2, also known as B7-DC)[3] (Figure 2).

The PD-1 receptor is expressed on the surface of the activated T lymphocyte, causing its exhaustion and, similarly to CTLA-4, PD-1 is expressed at high levels as well on the surface of Treg lymphocytes[3]. Unlike CTLA-4, PD-1 expression can be induced not only on the T cell surface, but also on other subsets of activated lymphocytes, such as B and NK cells[11], being widespread on a higher number of different immunologic actors. Therefore, the PD-1 blockade increases the NK cell cytotoxicity within the tumor, as well as the antibody activity mediated by the B lymphocytes expressing PD-1.

This immune-checkpoint is particularly relevant in inducing a state of exhaustion or anergy among the specific T lymphocytes chronically exposed to the antigen, as in the case of chronic viral infections and cancer. Therefore, the PD-1 blockade has a role in the reinvigoration of the anergic effector cells, blocking the immunological exhaustion provoked by chronic antigenic stimulation[3].

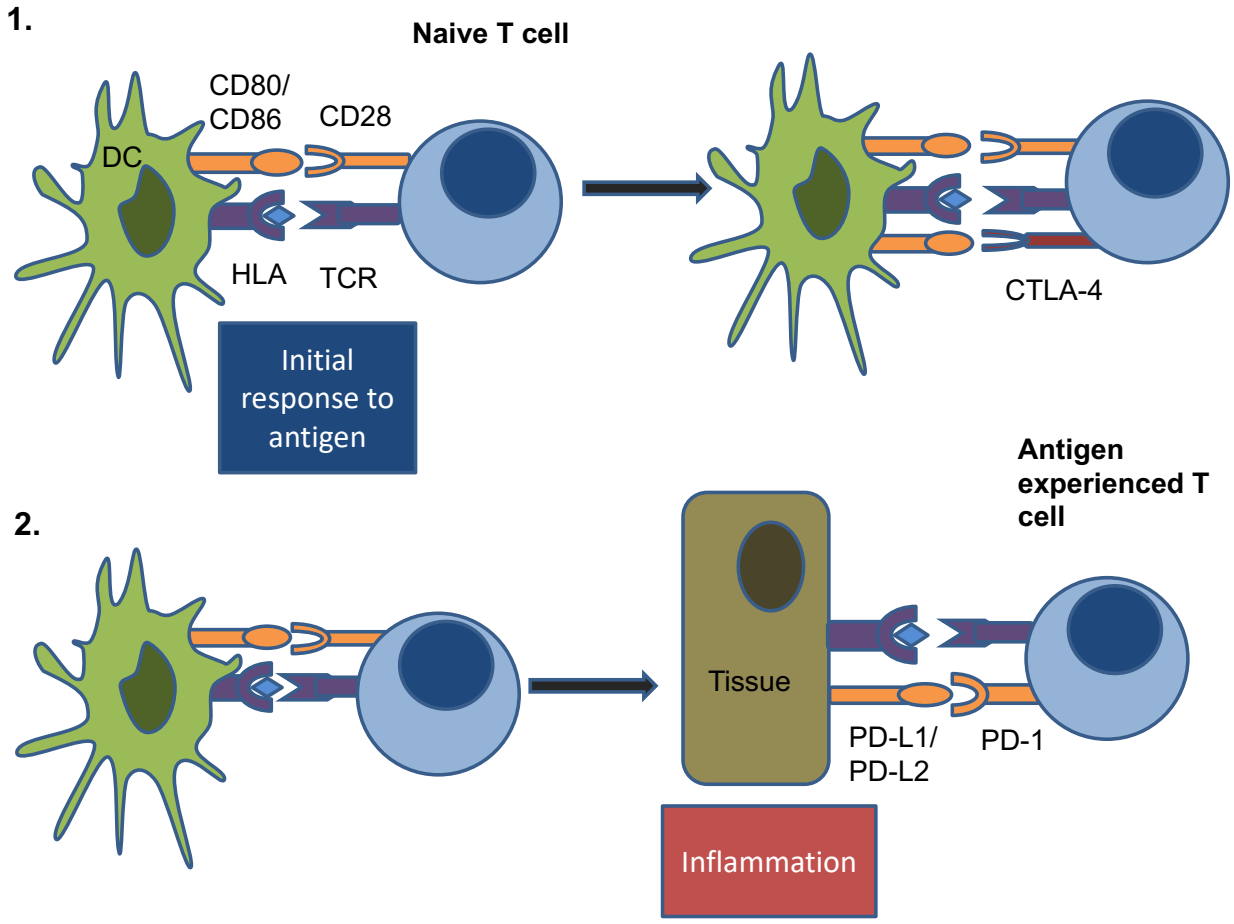

Figure 1. Distinct mechanism of the CTLA-4 and PD-1 immune checkpoints.

1. CTLA-4 blocks the co-stimulatory link between the CD28 receptor, expressed on the surface of the naïve T cells, and the CD80/CD86 molecules, during T cell activation, leading to an inhibitory signal.

2. On the contrary**,** the PD-1 receptor is expressed on the surface of effector T cells, playing a role in the peripheral site of the immune response, its expression is directly induced by the inflammatory response itself. Actually, during the chronic antigen exposure states, PD-1 can lead to the T cells exhaustion.

Thanks to these preclinical bases, the fully human IgG4 anti-PD-1 nivolumab and the fully humanized IgG4 anti-PD-1 pembrolizumab achieved revolutionary outcomes in clinical trials leading to their approval by the regulatory agencies in 2014.

In the randomized controlled phase 3 clinical study CheckMate 037, nivolumab 3 mg/kg every two weeks was compared to an investigator choice chemotherapy (ChT) in 370 melanoma advanced disease patients, pretreated with ipilimumab or BRAF inhibitors. The primary endpoint was the objective response rate (ORR), which was higher in the

nivolumab arm: 31.7% and 10.6%, respectively. Concerning the toxicity rate, fatigue, pruritus and diarrhea were the most observed toxicities in the nivolumab arm. Furthermore, grade 3-4 (G3-4) toxicity was observed in 9% of patients in the nivolumab *vs*. 31% in the ChT arm, and, in particular, the main G3-4 toxicities observed in the nivolumab arm were increased lipase, increased alanine aminotransferase, fatigue and anemia[12]. Nivolumab was also studied in a first-line setting, compared with DTIC in BRAF wild-type melanoma patients, in the phase 3 clinical study CheckMate 066. The primary endpoint was OS. The 3-year OS was 51.2% *vs.* 21.6% in the nivolumab and the DTIC arms, respectively. The main irAEs were diarrhea and elevated alanine aminotransferase level[13,14].

The randomized phase 3 clinical trial KEYNOTE-006 compared the efficacy of pembrolizumab *vs.* ipilimumab in checkpoint-inhibitor-naive melanoma advanced disease patients. The study randomized 834 patients in 1:1:1 to receive pembrolizumab 10 mg/kg every two weeks or every three weeks or four doses of ipilimumab 3 mg/kg every three weeks. Pembrolizumab was administered until disease progression, unacceptable toxicity or 24 months of therapy. Furthermore, patients with confirmed complete responses who received pembrolizumab for at least 6 months could discontinue therapy after receiving two cycles following the achievement of confirmed complete response. The primary endpoints were OS and progression free survival (PFS). In the 4-years update, presented at ASCO 2018, the OS data for treatment-naive patients were 44.3% for patients who received pembrolizumab *vs.* 36.4% in the ipilimumab arm, with a 4-years PFS of 36.2% for pembrolizumab *vs.*15.9% for ipilimumab[15,16]. Concerning adverse events, the most common observed AE in the pembrolizumab arms were fatigue, diarrhea, rash and pruritus. While the most frequently observed irAEs in the pembrolizumab arms were hypothyroidism (10.1% and 8.7% in the q14 and q21 schedules, respectively), hyperthyroidism (6.5% and 3.2%), of which G3-4 were mainly colitis (1.4% and 2.5%) and

hepatitis (1.1% and 1.8%). In the ipilimumab arm the most observed irAE was colitis (8.2%), while the main G3-4 were colitis (7.0%) and hypophysitis (1.6%)[17].

In this clinical trial, the possibility of discontinuing an immune-checkpoint inhibitor after 24 months of treatment has been studied, enhancing the concept of the achievement of the immunological memory leading to sustained and prolonged objective responses over time.

See Table 1[9,12,14,17,18] for a summary of data from the phase 3 clinical trials of immunotherapy in melanoma.

Study	**Treatment**	**ORR (%)**	**PFS (months)**	**3-year OS (%)**	**Grade 3/4 AEs (%)**
CA184-024[9]	Ipilimumab (10 mg/kg) + DTIC	15.2	3	20.8	56.3
	vs.	10.3	3	12.2	27.5
	DTIC				
KEYNOTE-006[17]	Pembrolizumab vs.	36-37	5.6-4.1	48.1	10.1-13-3
	Ipilimumab (3 mg/kg)	13	2.8	37.8	19.9
CheckMate 037[12]	Nivolumab vs.	31.7	3.1	-	9
	ChT	10.6	3.7		34
CheckMate 066[14]	Nivolumab vs.	40	5.1	51.2	34
	DTIC	14	2.2	21.6	38
CheckMate 067[18]	Nivolumab +	58	11.5	58	59
	ipilimumab vs.	44	6.9	52	21
	Nivolumab vs.	19	2.9	34	28

	Ipilimumab (3 mg/kg)	

Table 1. Overview of the principal immunotherapy clinical trials in metastatic melanoma.

Combination of Immune-checkpoint inhibitors

The encouraging preclinical and clinical results obtained with immune-checkpoint inhibitors as single agents have led the investigators to study the possibility of combining immunotherapeutic agents with different mechanisms of action.

In particular, the phase 3, clinical study CheckMate 067 randomized 1:1:1 a total of 945 naive patients with advanced melanoma, stratified by BRAF mutational status, AJCC M stage, and PD-L1 tumor expression, to receive nivolumab 1 mg/kg + ipilimumab 3 mg/kg every three weeks for 4 doses then nivolumab 3 mg/kg every two weeks, or nivolumab 3 mg/kg every two weeks + ipilimumab-matched placebo, or ipilimumab 3 mg/kg every three weeks for 4 doses + nivolumab-matched placebo. The primary endpoints were PFS and OS compared between the arms containing nivolumab and the ipilimumab arm.

The 4-years data showed a PFS of 37%, 31% and 9%, in the nivolumab + ipilimumab, nivolumab and ipilimumab arms, respectively. The 4-years OS were 53%, 46% and 30%, in the nivolumab + ipilimumab, nivolumab and ipilimumab arms, respectively[19]. Even if the study was not designed to statistically demonstrate the superiority of the nivolumab + ipilimumab regimen *vs.* nivolumab alone, the RR, PFS and OS of the combination regimen appeared superior to those of nivolumab alone. In addition, CheckMate 067 is the second phase 3 clinical trial after the KEYNOTE-006 trial which confirms the superiority of an anti-PD-1 *vs.* an anti-CTLA-4.

The patients stratification based on the level of intratumoral expression of PD-L1 showed that the immuno-combination therapy gives an advantage of about 10% over the nivolumab monotherapy in terms of OS, when the PD-L1 expression level is < 1%, while the OS curves are substantially overlapping when the PD-L1 level is ≥1%.

However, these results were obtained at the expense of a high toxicity profile. In fact, 59% of patients who received nivolumab + ipilimumab showed G3-4 AEs, *vs.* 22% and 28 % in

the nivolumab and ipilimumab arms, respectively. The most important G3 AEs were diarrhea and colitis in all treatment arms[18,19].

Based on these results, the nivolumab + ipilimumab combination has been approved by the regulatory authorities.

In order to overcome the obstacle of the high toxicity profile, the investigators evaluated in a phase 1 study pembrolizumab + low dose ipilimumab (1 mg/kg) for four cycles every three weeks, followed by pembrolizumab alone. This combination showed an incidence of G3-4 AEs of 42% and an ORR of 57%[20].

CheckMate 511 was a phase 3b/4 trial conducted to determine if nivolumab 3 mg/kg + ipilimumab 1 mg/kg (NIVO3+IPI1) had a better toxicity profile than the approved nivolumab 1 mg/kg + ipilimumab 3 mg/kg (NIVO1+IPI3) combination. The primary endpoint was the incidence of treatment-related G3-G5 AEs in the two arms. Incidence of G3-5 AEs was significantly lower in the NIVO3+IPI1 arm compared with NIVO1+IPI3 (34% *vs.* 48%; p=0.006).[21] Despite the study was not designed to demonstrate the noninferiority of NIVO3+IPI1 to NIVO1+IPI3 for efficacy, the observed ORR was 45.6% in the NIVO3+IPI1 arm *vs.* NIVO1+IPI3 arm, with a median PFS of 9.9 months in the NIVO3+IPI1 arm and 8.9 months in the NIVO1+IPI3 arm[21].

The phase 3 clinical trial KEYNOTE-252 compared the efficacy of pembrolizumab *vs.* pembrolizumab + epacadostat, an indoleamine 2,3-dioxygenase (IDO) enzyme inhibitor, implicated in the mechanisms of immunotolerance and immunoescape. However, despite the promising results of phase 1-2, the 1-year observed PFS and OS in the two arms were similar[22]. Other combinations of anti-PD-1 plus the immune-checkpoint lymphocyte activation gene-3 (LAG-3) inhibitor are under evaluation.

Finally, the Part 3 of the clinical study KEYNOTE-022 data have been recently presented. A total of 120 naïve melanoma advanced disease patients bearing the *BRAF* V600E/K mutation, were randomized to receive dabrafenib + trametinib + pembrolizumab 2 mg/kg

every three weeks or dabrafenib + trametinib + placebo, even if the two arms were not balanced in terms of different stage IV subtypes with prevalence of M1c in the experimental arm with respect to the placebo arm (82% *vs.* 63%). The primary endpoint was PFS, which was numerically higher in the dabrafenib + trametinib + pembrolizumab arm (1-year PFS 59% *vs.* 45%) but did not reach the established threshold of significance as per study design. In the experimental arm, even if such difference was not statistically significant, more CRs were observed (18.3% *vs.* 13.3%), with a greater number of G3-4 AEs (57% *vs.* 27% treatment-related AEs)[23]. It should be noted, however, that 50% of patients in the placebo arm received immunotherapy at disease progression. A more extended follow-up could clarify whether the initial differences seen in PFS and response duration will be confirmed.

Brain metastasis

The presence of brain metastases is associated with poor prognosis in advanced melanoma patients, and they still represent a crucial therapeutic challenge. Furthermore, brain metastases have been an exclusion criteria from the majority of clinical studies[24]. Recently, two clinical studies investigated the efficacy of the combination of nivolumab + ipilimumab in these patients.

The CheckMate 204 trial is a phase 2 clinical trial which included patients with at least one ≥5 mm, non-irradiated brain metastasis, in absence of neurological symptoms. Patients received nivolumab 1 mg/kg + ipilimumab 3 mg/kg in the induction phase every three weeks for four cycles, followed by nivolumab 3 mg/kg every two weeks. The primary endpoint was the intracranial clinical benefit (an endpoint consisting of CR, PR and SD for more than 6 months). The observed intracranial response rate was 57% (with a 26% of CR). After one year, the observed PFS and OS were 60% and 82%, respectively. G3-4 AEs were reported in 55% of patients[25]. The experimental therapy has therefore shown good local and systemic disease control.

The ABC (Anti-PD-1 Brain Collaboration) Trial is another phase 2 clinical trial with similar inclusion criteria to CheckMate 204, but included three cohorts: asymptomatic patients were randomized or in Cohort A, receiving nivolumab 1 mg/kg + ipilimumab 3 mg/kg every three weeks for four cycles, followed by nivolumab 3 mg/kg every two weeks, or in Cohort B, receiving nivolumab 3 mg/kg every two weeks. While, locally pretreated, symptomatic, or with leptomeningeal involvement, received instead nivolumab 3 mg/kg every two weeks. The primary endpoint was the intracranial response at week 12. The Cohort A showed intracranial ORR comparable to that observed in CheckMate 204. While patients who received nivolumab monotherapy showed, in Cohort B (asymptomatic) an ORR of 21%, and in the Cohort C (symptomatic) an ORR of 6%[26]. This study confirmed as well that the

combination of nivolumab + ipilimumab should be considered as a first-line therapy for patients with untreated and asymptomatic melanoma brain metastases.
However, these encouraging data does not involve patients with symptomatic brain metastases yet. Further studies are needed to investigate immunotherapy in this subset of patients.

The pursuit of a biomarker

Immune-checkpoint inhibitors and in particular the anti-PD-1 agents showed an important antitumor effect with increased ORRs and an improved survival in melanoma and multiple other cancers patients. However, not all patients respond to such treatment, and in order to understand if there is a way to predict which patients will respond to immune-checkpoint inhibitors, many biomarkers have been tested.
From a clinical point of view, the most important, predictive biomarker is serum lactate dehydrogenase (LDH). High baseline levels of serum LDH have been associated to a less favorable outcome in terms of response rate, OS and PFS in patients treated both with targeted therapy (3-years OS 55% *vs.* 22% in normal and elevated serum LDH, respectively)[27], and immunotherapy with an anti-PD-1 (4-years OS 54% *vs.* 31% in normal and elevated serum LDH, respectively) and with an anti-CTLA-4 (4-years OS 38% *vs.* 17% in normal and elevated serum LDH, respectively)[19].
In particular, the most important described biomarkers can be classified into two categories, as illustrated by Cristescu in a recent article analyzing the predictive biomarkers of response to pembrolizumab: (1) those related to tumor neoantigen burden, such as the microsatellite instability high/deficient mismatch repair (MSI-H/dMMR) or high tumor mutational burden (TMB); and (2) those related to T cell-inflammed tumor

microenvironment (TME), such as the expression by tumor cells and immune system cells of PD-L1 at different levels, and the gene signatures of activated T cells.

In a pan-tumor cohort, it was observed that high levels of both TMB and T cell-inflammed tumor gene expression profile (GEP) are predictive of the response to pembrolizuamb and give significant advantage in terms of PFS[28].

On this basis, the U.S. Food and Drug Administration (FDA) has approved the use of nivolumab for the treatment of certain cancers (such as metastatic colorectal cancer) having MSI-H/dMMR, and pembrolizumab for any solid tumor with MSI-H/dMMR.

Concerning the immunogenicity of tumors, Galon and colleagues suggested that it is essential to understand the mechanisms underlying hot, altered and cold tumors in order to use the correct therapy for the right tumor, activate the specific immune response and convert immune cold tumors into immune hot[29]. Hot tumors present a high T specific lymphocyte infiltrate, even if mainly with an exhausted phenotype expressing high levels of inhibitory receptors, and in particular CTLA-4 and PD-1. For this reason, they represent the best substrate for therapy with immune-checkpoint inhibitors, as single agents or in combination with each other. Based on preclinical data, chronic interferon response can lead to the immune-checkpoint inhibitors resistance, while the use of co-stimulatory molecules, although promising, is clinically limited by the high toxicity profile.

In addition to the use of immune-checkpoint inhibitors, recent studies showed how microbiota can influence the development of a hot tumor microenvironment. For example, mice receiving fecal microbiota transplantation from immune-checkpoint inhibitors responder patients presented an upregulation of PD-L1[30]. In excluded tumors, instead, there is an accumulation of T CD8 lymphocytes around the tumor, without an actual infiltrate. The host is able to mount a specific immune response, but there is physical inability to reach the tumor. This disability may be due to a lack of chemokines resulting from modulation of genetic and epigenetic pathways responsible of their expression. For

example, DNA methylation can reduce the levels of CXCL9 and CXCL10 in some tumors[31,32]. Another reason could be the barrier effect resulting from an ineffective tumor vascularization, which could benefit from the combination of immune-checkpoint inhibitors with anti-angiogenic agents[33].

Another reason for the failure to develop an adequate adaptive immune response is the failure to activate the innate response. For this reason, the antineoplastic potential of Toll-like receptors (TLR) has been studied. In particular, the intratumoral injection of TLR9 agonist in combination with immune-checkpoint inhibitors[34,35].

Cold tumors are associated with poor prognosis. The proposed strategy to convert them into hot tumors is the combination of a priming phase that activates the specific T lymphocyte response with, for example, vaccines, adoptive T cell transfer, with immune-checkpoint inhibitors or co-stimulatory molecules. Other approaches may include radiation therapy, chemotherapy, targeted therapies or oncolytic virus therapy[29].

Concluding remarks

The introduction of immune-checkpoint inhibitors, and in particular of anti-PD-1/PD-L1 agents, has not only revolutionized the therapeutic paradigm in melanoma, leading to long-lasting responses in almost half of patients, but also in an increasing number of tumors. However, clinical and translational research should focus on the non-responders subset of patients, in order to convert the immune-cold tumors into immune-hot ones. As suggested by Galon[29], the approach should be multimodal and based on a preclinical rationale, identifying the characteristics of the tumor immune infiltrate, the activation status of the adaptive and the innate response as well, and the ability of the tumor-specific lymphocytes to reach the tumor bed. Moreover, the immunological characterization should go along with the TMB characterization, in order to plan a personalized therapeutic strategy. The colder the tumor, the more different therapeutic approaches will be

needed[29]. However, even in hot tumors the anti-PD-1 monotherapy is not sufficient to achieve the maximal efficiency.

Further studies are still needed to identify the role of the microbiota in antitumor immunity, especially from a clinical-practice point of view. Finally, more efforts should be addressed to understand which is the most appropriate approach for the treatment of symptomatic brain metastases.

References

1 Rosenberg SA, Lotze MT, Yang JC, *et al.* Prospective randomized trial of high-dose interleukin-2 alone or in conjunction with lymphokine-activated killer cells for the treatment of patients with advanced cancer. *J Natl Cancer Inst* 1993; **85**: 622–32.

2 Marconcini R, Spagnolo F, Stucci LS, *et al.* Current status and perspectives in immunotherapy for metastatic melanoma. *Oncotarget* 2018; **9**: 12452–70.

3 Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012; **12**: 252–64.

4 Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003; **299**: 1057–61.

5 Barzaghi F, Passerini L, Bacchetta R. Immune dysregulation, polyendocrinopathy, enteropathy, x-linked syndrome: a paradigm of immunodeficiency with autoimmunity. *Front Immunol* 2012; **3**: 211.

6 Ribas A. Clinical development of the anti-CTLA-4 antibody tremelimumab. *Semin Oncol* 2010; **37**: 450–4.

7 Hodi FS, O'Day SJ, McDermott DF, *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010; **363**: 711–23.

8 McDermott D, Haanen J, Chen T-T, Lorigan P, O'Day S, MDX010-20 Investigators. Efficacy and safety of ipilimumab in metastatic melanoma patients surviving more than 2 years following treatment in a phase III trial (MDX010-20). *Ann Oncol Off J Eur Soc Med Oncol* 2013; **24**: 2694–8.

9 Robert C, Thomas L, Bondarenko I, *et al.* Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med* 2011; **364**: 2517–26.

10 Ascierto PA, Del Vecchio M, Robert C, *et al.* Ipilimumab 10 mg/kg versus ipilimumab 3 mg/kg in patients with unresectable or metastatic melanoma: a randomised, double-blind, multicentre, phase 3 trial. *Lancet Oncol* 2017; **18**: 611–22.

11 Sivori S, Vacca P, Del Zotto G, Munari E, Mingari MC, Moretta L. Human NK cells: surface receptors, inhibitory checkpoints, and translational applications. *Cell Mol Immunol* 2019; **16**: 430–41.

12 Weber JS, D'Angelo SP, Minor D, *et al.* Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol* 2015; **16**: 375–84.

13 Ascierto PA, Long GV, Robert C, *et al.* Survival Outcomes in Patients With Previously Untreated BRAF Wild-Type Advanced Melanoma Treated With Nivolumab Therapy: Three-Year Follow-up of a Randomized Phase 3 Trial. *JAMA Oncol* 2018; published online Oct 25. DOI:10.1001/jamaoncol.2018.4514.

14 Robert C, Long GV, Brady B, *et al.* Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med* 2015; **372**: 320–30.

15 Schachter J, Ribas A, Long GV, *et al.* Pembrolizumab versus ipilimumab for advanced melanoma: final overall survival results of a multicentre, randomised, open-label phase 3 study (KEYNOTE-006). *Lancet Lond Engl* 2017; **390**: 1853–62.

16 Long GV, Schachter J, Ribas A, *et al.* 4-year survival and outcomes after cessation of pembrolizumab (pembro) after 2-years in patients (pts) with ipilimumab (ipi)-naive advanced melanoma in KEYNOTE-006. *J Clin Oncol* 2018; **36**: 9503–9503.

17 Robert C, Schachter J, Long GV, *et al.* Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N Engl J Med* 2015; **372**: 2521–32.

18 Wolchok JD, Chiarion-Sileni V, Gonzalez R, *et al.* Overall Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma. *N Engl J Med* 2017; **377**: 1345–56.

19 Hodi FS, Chiarion-Sileni V, Gonzalez R, *et al.* Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. *Lancet Oncol* 2018; **19**: 1480–92.

20 Long GV, Atkinson V, Cebon JS, *et al.* Standard-dose pembrolizumab in combination with reduced-dose ipilimumab for patients with advanced melanoma (KEYNOTE-029): an open-label, phase 1b trial. *Lancet Oncol* 2017; **18**: 1202–10.

21 Lebbé C, Meyer N, Mortier L, *et al.* Evaluation of Two Dosing Regimens for Nivolumab in Combination With Ipilimumab in Patients With Advanced Melanoma: Results From the Phase IIIb/IV CheckMate 511 Trial. *J Clin Oncol Off J Am Soc Clin Oncol* 2019; **37**: 867–75.

22 Long GV, Dummer R, Hamid O, *et al.* Epacadostat (E) plus pembrolizumab (P) versus pembrolizumab alone in patients (pts) with unresectable or metastatic melanoma: Results of the phase 3 ECHO-301/KEYNOTE-252 study. *J Clin Oncol* 2018; **36**: 108–108.

23 Ascierto, A., Ferrucci PF, Ribas, A. KEYNOTE-022 Part 3: Phase II randomized study of 1L dabrafenib (D) and trametinib (T) plus pembrolizumab (Pembro) or placebo (PBO) for BRAF-mutant advanced melanoma. 2018. https://oncologypro.esmo.org/Meeting-Resources/ESMO-2018-Congress/KEYNOTE-022-Part-3-Phase-2-Randomized-Study-of-1L-Dabrafenib-D-and-Trametinib-T-Plus-Pembrolizumab-Pembro-or-Placebo-PBO-for-BRAF-Mutant-Advanced-Melanoma.

24 Gershenwald JE, Scolyer RA, Hess KR, *et al.* Melanoma staging: Evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual: Melanoma Staging: AJCC 8 th Edition. *CA Cancer J Clin* 2017; **67**: 472–92.

25 Tawbi HA, Forsyth PA, Algazi A, *et al.* Combined Nivolumab and Ipilimumab in Melanoma Metastatic to the Brain. *N Engl J Med* 2018; **379**: 722–30.

26 Long GV, Atkinson V, Lo S, *et al.* Combination nivolumab and ipilimumab or nivolumab alone in melanoma brain metastases: a multicentre randomised phase 2 study. *Lancet Oncol* 2018; **19**: 672–81.

27 Schadendorf D, Long GV, Stroiakovski D, *et al.* Three-year pooled analysis of factors associated with clinical outcomes across dabrafenib and trametinib combination

therapy phase 3 randomised trials. *Eur J Cancer* 2017; **82**: 45–55.

28 Cristescu R, Mogg R, Ayers M, *et al.* Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. *Science* 2018; **362**. DOI:10.1126/science.aar3593.

29 Galon J, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. *Nat Rev Drug Discov* 2019; **18**: 197–218.

30 Gopalakrishnan V, Spencer CN, Nezi L, *et al.* Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* 2018; **359**: 97–103.

31 Nagarsheth N, Peng D, Kryczek I, *et al.* PRC2 Epigenetically Silences Th1-Type Chemokines to Suppress Effector T-Cell Trafficking in Colon Cancer. *Cancer Res* 2016; **76**: 275–82.

32 Peng D, Kryczek I, Nagarsheth N, *et al.* Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. *Nature* 2015; **527**: 249–53.

33 Tian L, Goldstein A, Wang H, *et al.* Mutual regulation of tumour vessel normalization and immunostimulatory reprogramming. *Nature* 2017; **544**: 250–4.

34 Sato-Kaneko F, Yao S, Ahmadi A, *et al.* Combination immunotherapy with TLR agonists and checkpoint inhibitors suppresses head and neck cancer. *JCI Insight* 2017; **2**. DOI:10.1172/jci.insight.93397.

35 Wang S, Campos J, Gallotta M, *et al.* Intratumoral injection of a CpG oligonucleotide reverts resistance to PD-1 blockade by expanding multifunctional CD8+ T cells. *Proc Natl Acad Sci U S A* 2016; **113**: E7240–9.